Normal Incorporation of Oral Iron into Intestinal Ferritin in Inflammation[1] (36200)

STANLEY G. SCHADE
(Introduced by Paul Heller)

Department of Medicine, University of Illinois, Abraham Lincoln School of Medicine and Veterans Administration West Side Hospital, Chicago, Illinois

Inflammation caused by turpentine abscesses reduces the intestinal absorption of iron (1). During iron absorption a variable quantity of iron entering the intestinal cell appears to be sequestered in the cell and reenters the intestinal lumen when the cell dies (2, 3). Crosby has postulated that this iron is sequestered in the form of ferritin and that this process serves the regulation of iron absorption by preventing the transfer of iron from the intestinal cell to the body of the animal (4). According to this theory an increased fraction of iron in the intestinal wall (intestinal iron) should be found in ferritin in conditions in which iron absorption is decreased. The present study, therefore, was designed to examine the hypothesis that inflammation causes an increased proportion of iron entering the intestinal mucosa to be incorporated into ferritin and that this iron is not transferred into the body. The results suggest that the decreased absorption of iron in inflammation is indeed associated with a defect in the transfer of iron from the intestinal cell to the body, but that this defect is not the result of increased sequestration of iron in intestinal ferritin.

Methods. Absorption studies were done in 200–300 g male Holtzman rats which were fasted overnight. The rats were given intragastric doses of iron under ether anesthesia with a 17-gauge olive-tipped endoesophageal needle. The test dose contained 3–5 μCi of ^{59}Fe with a carrier dose of 5 μmol of iron as $FeCl_2 \cdot 4H_2O$. Twenty-four hours prior to dosing 0.2 ml of turpentine was injected into the thigh muscle to produce inflammation in the experimental animals.

After administration of iron each rat was placed in a vented quart container and whole body radioactivity was measured in a small animal whole body liquid scintillation detector (Armac, Packard). At periodic intervals after dosing the animals were sacrificed, the intestines were excised from esophagus to rectum, washed in distilled water, and frozen. Radioactivity in the intestine and the carcass was then measured in the small animal detector. By using the specific activity of the test dose, results were converted to nanomoles of iron.

Incorporation of iron into the ferritin of the rat intestine was measured by immunologic precipitation of ferritin (5). Each intestine was homogenized in 40 ml of 0.1% (w/v) sodium azide in a Virtis tissue homogenizer. The homogenates were heated at 70° for 5 min, centrifuged at 12,000*g* for 10 min, and the supernates filtered through Whatman No. 1 paper. Duplicate 2 ml aliquots from the supernates were mixed with 0.5 ml of serum from rabbits which had been immunized with rat ferritin prepared by the method of Drysdale and Munro (6). A control specimen of 2 ml of supernate without immune serum was included. The samples were refrigerated at 4° for 12 hr and centrifuged at 12,000*g* for 10 min, and the precipitates were washed in normal saline. The precipitates were dissolved in 4 ml of 0.5 *N* NaOH. Radioactivity in the dissolved precipitates was measured in a gamma detector. The percentage of ^{59}Fe in ferritin was calculated from the net radioactivity in the precipitates and the net radioactivity in an aliquot of intestinal homogenate.

Results. The amounts of orally administered iron found in intestine and carcasses of

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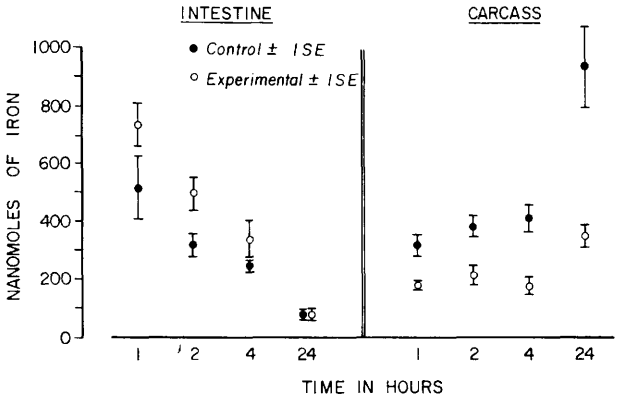

FIG. 1. Amount of iron found in the intestine and carcass of rats with turpentine abscesses and controls at intervals after the oral administration of 5 μmole of iron.

the animals at each interval are given in Fig. 1. At each interval studied the amount of orally administered iron found in the carcasses of the experimental animals was less than that found in the control animals. The quantity of iron in the intestines of the experimental animals was greater than that in the control animals except at 24 hr. The relative amount of iron in the intestine compared with that in the carcass was always greater in the experimental animals than in the control animals.

The percentage of ^{59}Fe in the intestinal homogenates found in the ferritin fraction is given in Table I. This percentage was no greater in the experimental animals than in the control animals. The percentage of intestinal iron in ferritin at one hour was significantly less in the experimental animals than in the control animals.

Discussion. The results of the present study show that in inflammation there is little or no decrease in the ability of iron to enter the intestinal cell from the intestinal lumen. Transfer of iron from the intestinal cell to the body of the animal is, however, depressed. These findings are similar to those described in animals treated with endotoxin which causes changes in iron metabolism similar to those seen in inflammation induced by turpentine abscesses (7, 8).

Although the quantity of intestinal iron found in the experimental animals was increased over that found in the control animals, the fraction of this iron found in ferritin was not increased. At one hour the fraction of iron in intestinal ferritin was even less in the experimental animals than in the control animals. At this time the same absolute amount of iron was in the intestinal ferritin of both groups of animals. This suggests that incor-

TABLE I. Percentage of Intestinal ^{59}Fe Found in the Ferritin Fraction of Intestinal Homogenate at Various Intervals after Oral Administration of ^{59}Fe.[a]

Time (hr)	Group	No. of Animals	% of intestinal Fe in intestinal ferritin	*P* value
1	control	7	42.6 ± 6.2	<.02
	experimental	7	30.0 ± 3.8	
2	control	13	62.9 ± 3.9	<.3
	experimental	13	67.2 ± 2.2	
4	control	14	63.7 ± 7.7	>.5
	experimental	14	60.3 ± 4.3	
24	control	6	30.3 ± 4.4	>.5
	experimental	7	30.9 ± 4.0	

[a] The values are given as the mean ± 1 SE with *P* value determined by Student's *t* tests.

poration of iron into the intestinal ferritin was proceeding at the same rate in both groups even though more iron was present in the intestines of the experimental group. Therefore, inflammation does not appear to reduce the transfer of iron from the intestinal cell to the body of the animal through enhanced sequestration of iron in the form of ferritin in the intestine. It seems more likely that incorporation of iron into ferritin occurs as a secondary response to the presence of iron and not as the primary cause of the defect of iron absorption in inflammation. A similar conclusion may be drawn from the report of Torrance *et al.* (8), stating that in animals given endotoxin a smaller fraction of intestinal iron is found in ferritin than in control animals.

Britten and Raval (9) have studied the role of ferritin in another condition in which iron absorption is reduced. In their study a reduction in absorption of iron by iron-deficient rats was produced by giving the animals 1 mg of iron intraduodenally five hours prior to the test dose. Although absorption was reduced from 25% to 14%, only 0.5% of the second dose was found in duodenal ferritin. The present study supports their argument that the role of ferritin is not to prevent iron absorption by binding iron in the intestinal mucosa.

The nature of the defect in the transfer of iron from the intestinal cell to the body remains obscure. Since inflammation inhibits the release of iron from both the reticuloendothelial cell (10) and the intestinal cell, it seems likely that a similar mechanism is present in both cells.

Summary. Inflammation in rats caused by turpentine abscesses depresses iron absorption by inhibiting the transfer of iron from the intestinal mucosa to the body of the animal. It has been postulated that this inhibition is due to increased sequestration of orally administered iron in intestinal ferritin. The results of the described experiments show that this is not the case and provide further evidence that ferritin does not prevent iron absorption by binding iron in the intestinal mucosa.

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